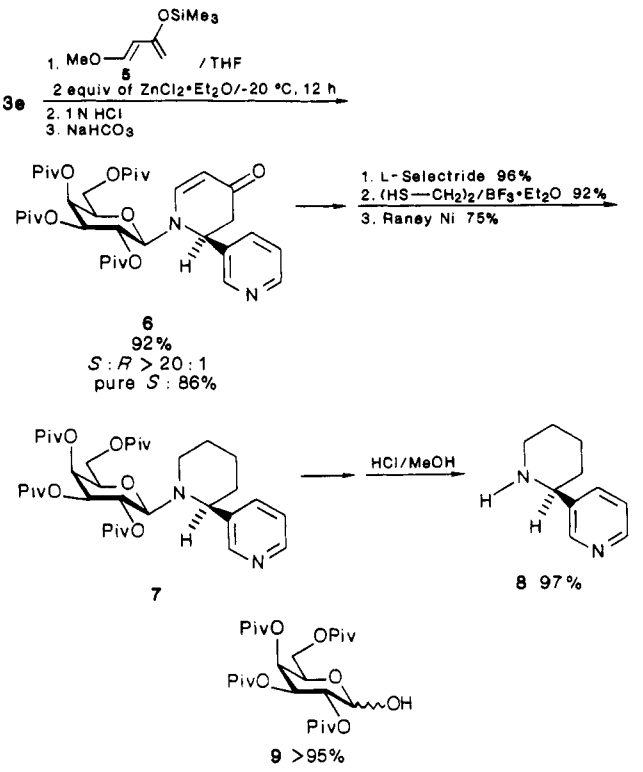
Scheme III



and extraction with dichloromethane, the *N*-galactosyldehydropiperidinone derivative **6** was isolated in high yield showing a diastereomeric ratio of more than 20:1 (HPLC and 400-MHz ^{1}H NMR). The pure diastereomer **6**[10] was isolated by flash chromatography in about 86% yield (Scheme III).

Compound **6** is then converted into the *N*-galactosylanabasin **7** by reduction of its double bond with L-Selectride,[11] formation of the dithiolane derivative, and its desulfurization with Raney nickel. The final release of the anabasin **8** from the carbohydrate template is achieved almost quantitatively with HCl/methanol. For characterization, **8** is transformed into its *p*-nitrobenzoate.[12] Comparison of the optical rotation with that reported in the literature[13] demonstrated that the (*S*)-anabasin **8** is obtained. Consequently, the (*S*)-diastereomer of **6** is formed in the described cyclocondensation with high diastereoselectivity. During work up the O-pivaloylated galactose **9** can be isolated almost quantitatively and reconverted into the starting auxiliary **1** by a simple sequence of reactions.[2]

In conclusion, the carbohydrate template **1** offers a new and effective method for diastereoselective aza-Diels–Alder synthesis of interesting chiral nitrogen heterocycles. It should be noted that aza-Diels–Alder reactions in asymmetric form have been described only in a few isolated cases.[5,8b,14]

(10) Mp 176 °C; $[\alpha]^{20}_D$ = +19.6° (*c* = 3, $CHCl_3$).

(11) An analogous reaction on uraciles served as the model: Hannon, S. J.; Kundu, N. G.; Herzberg, R. P.; Bhatt, R. S.; Heidelberger, C. *Tetrahedron Lett.* **1980**, *21*, 1105.

(12) **8**, *p*-nitrobenzoate: mp 122 °C; $[\alpha]^{20}_D$ = −130.8° (*c* = 1.2, MeOH) (lit.[10] mp 127–128 °C); $[\alpha]^{20}_D$ = −130.0° (*c* = 3, MeOH).

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(14) For diastereoselective hetero-Diels–Alder reactions of chiral nitroso compounds with dienes, see: Felber, H.; Kresze, G.; Braun, H.; Vasella, A. *Tetrahedron Lett.* **1984**, *25*, 5381.

Enzyme-Catalyzed Enantioconvergent Lactonization of γ-Hydroxy Diesters in Organic Solvents

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Summary: A strategy has been developed for the enantioconvergent lactonization of symmetrical hydroxy diesters which exploits the prochiral stereospecificity of lipases in organic solvents. Using this approach, prochiral γ-hydroxypimelate diesters (**2**) were converted into either enantiomer of γ-butyrolactone γ-3-propionates (**3**).

Sir: It is now well established that hydrolytic enzymes can act as catalysts in anhydrous organic solvents,[1] where they catalyze ester synthesis and ester exchange rather than hydrolysis. Following the pioneering work of Klibanov,[2] several lipases (triacylglycerol hydrolases EC 3.1.1.3) have been used for the preparative resolution of chiral acids and alcohols via enantiospecific esterification and transesterification.[3] We recently found that porcine pancreatic lipase suspended in organic solvents catalyzes the stereospecific lactonization of esters of γ- and δ-hydroxy carboxylic acids, and we have used this method to prepare gram quantities of optically pure substituted γ- and δ-lactones.[4,5] A similar approach was developed by Yamada[6] and by Sih[7] for the preparation of macrocyclic lactones under carefully controlled kinetic conditions.

All of these experiments rely on the enantiospecificity of the enzymatic conversions and as such amount to kinetic resolutions of racemic hydroxy esters. The theoretical yield of chiral lactone from such a reaction is 50%, although in practice it will be considerably lower. This is because competitive lactonization of the unwanted enantiomer increases as the reaction progresses, so that the reaction must be stopped at low conversion to optimize optical purity. This problem does not arise if the substrate is prochiral. Enzymes exhibit prochiral stereospecificity, i.e. the ability to discriminate between enantiotopic groups of a prochiral molecule. In principle, the enzymic cyclization of a prochiral hydroxy diester will be enantiocon-

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Table I. Asymmetric Lactonization of γ-Hydroxypimelate Diesters with Lipases in Organic Solvents[a]

prochiral substrate	enzyme	time, h	convers,[b] %	chiral product[c]	$[\alpha]_D^{28}$ (c in CH_2Cl_2), deg	% ee
2b	PPL	44	100	*S*-**3b**	−60.86 (*c* 0.58)	>98[d]
2b	PF	44	100	*R*-**3b**	+6.61 (*c* 1.1)	32[d]
2c	PPL	68	33	*S*-**3c**	−26.85 (*c* 0.51)	46[d]
2c	PF	68	36	*R*-**3c**	+5.82 (*c* 0.8)	15[d]
2d[e]	PPL	52	100	*S*-**3d**	−40.86 (*c* 0.74)	>95

[a] The experimental protocol was the same as described in the text for **2b**. No lactonization took place in the absence of enzymes under the conditions used. [b] Reaction progress was monitored (to within ±5% accuracy) by periodic examination of aliquots from the reaction mixture by IR, comparing the 1770 cm^{-1} γ-lactone carbonyl absorption of the product and the 1730 cm^{-1} ester carbonyl absorption of the starting hydroxy diester; and by ^1H NMR, comparing the relative intensities of the C-4 protons in hydroxy diesters **2b–d** at 3.65 ppm and in the corresponding lactones **3b–d** at 4.48 ppm. [c] All the products are oils, which were purified by chromatography on preparative silica gel plates. Their identity was confirmed by IR and ^1H NMR and in the case of **3b** by comparison with literature data.[9d] [d] The optical purity determination of **2b–c** was based on a difference in the ^1H NMR chemical shifts in the two enantiomers for the methylene or methyne protons of the alcohol moiety in the presence of the chiral solvating reagent, (*R*)-(−)-2,2,2-trifluoro-1-(9-anthryl)ethanol, as previously described.[9d] [e] The substrate **2d** is poorly soluble in hexane, but the lactone **3d** is, so progressive dissolusion occurs as lactonization proceeds.

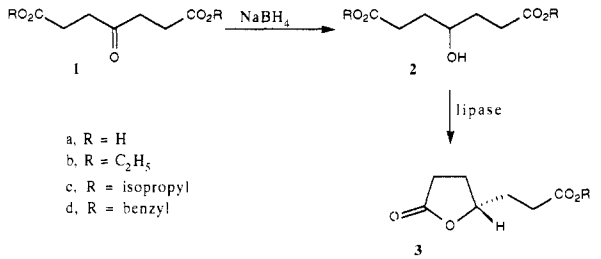

Figure 1.

vergent and will convert *all* of the precursor into a single enantiomer of the lactone.

In the present work we demonstrate this novel strategy for enzymes in organic solvents by the enantioconvergent lactonization of the prochiral symmetrical hydroxy diesters. γ-Hydroxypimelates **2** were selected as the model prochiral compounds and we hereby describe their conversion into the optically active γ-butyrolactone γ-3-propionates **3** (Figure 1). These lactones are versatile chiral building blocks in the synthesis of biologically active natural products,[8] but previous enantioselective syntheses have either required critical control of the reaction conditions or have given products of relatively low optical purity.[9] Our approach provides a convenient, high yielding, and enantioselective route to this class of compounds.

Since γ-hydroxypimelic acid itself, **2a**, undergoes spontaneous lactonization, we investigated the behavior of its diesters **2b–d**. These were obtained in nearly quantitative yields by $NaBH_4$ reduction of the corresponding keto diesters **1b–d**[10] and shown not to lactonize spontaneously. Screening of seven commercially available lipase and two protease preparations with **2b–d** in organic solvents revealed that porcine pancreatic lipase (PPL) and the lipase from *Pseudomonas fluorescens* (PF) catalyzed lactonization.[11]

For both enzymes lactonization was enantioconvergent, but the stereospecificity was strikingly different (Table I): PPL gave the *S* enantiomer with high enantiomeric excess, while PF gave the *R* enantiomer in lower optical yield. For PPL the selectivity was strongly dependent on the ester alcohol, the best results being obtained for the faster reacting ethyl and benzyl esters, and markedly lower enantiomeric excess with the more bulky isopropyl ester. Furthermore, palladium oxide catalyzed hydrogenation of the enzymatically produced benzyl ester **3d** in glacial acetic acid afforded crystalline (*S*)-(−)-lactonic acid **3a** with ee >95% in quantitative yield, $[\alpha]^{28}_D$ −39.86° (*c* 0.77, H_2O).[12] Possessing two readily distinguishable functional groups, optically pure lactonic acid **3a** provides a versatile chiral building block for the synthesis of biologically active natural products such as pheromones[13] and antifungal metabolites.[14]

In a typical enzymatic experiment 360 mg of the powdered enzyme preparation was added to 6 mL of a 100 mM solution of γ-hydroxy diethyl pimelate **2b** in hexane, and the suspension (enzymes are totally insoluble in hexane and nearly all other organic solvents[1]) was vigorously shaken at 40 °C at 200 rpm. Progress was followed spectrophotometrically as described in footnote *b* of Table I, and the reaction was terminated after 44 h by simply filtering off the enzyme. The lactone was purified by silica gel chromatography.

This study significantly extends the synthetic utility of lipases in organic solvents. Our procedure allows the en-

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The $NaBH_4$ reduction of keto diesters **1b–d** was carried out as in Nozaki, H.; Kikondo, K.; Nakanisi, O.; Sisiso, K. *Tetrahedron* **1963**, *19*, 1617. However, to avoid spontaneous lactonization the reduction was carried out at −10 °C and acidic conditions were rigorously avoided during workup. The hydroxy diesters **2b–d** were purified by silica gel chromatography, and their structures were confirmed by ^1H NMR and IR spectroscopy.

(11) Porcine pancreatic lipase was purchased from Sigma Chemical Co. as a powder with a specific activity of 11 units/mg solid. Lipase from the bacterium *Pseudomonas fluorescens* was kindly provided by Amano Pharmaceutical Co. The seemingly large amount of enzyme used in this work is misleading, for the commercial preparations employed are crude containing less than 1% total protein.

No lactonization of **2b–d** was observed with lipases from *Aspergillus, Mucor*, and *Rhizopus* (from Amano); lipases from *Candida cylindracea* and from wheat germ (Sigma); and with the proteases subtilisin Carlsberg from *Bacillus subtilis* and protease from *Streptomyces griseus* (Sigma).

(12) This value is considerably higher than that reported in ref 9a; $[\alpha]^{25}_D$ −26.5° (H_2O).

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antioconvergent transformation of an achiral substrate possessing σ-symmetry into a single enantiomer of a chiral lactone. Considering the broad substrate specificity of lipases, this approach is expected to be synthetically useful for asymmetric preparation of other optically pure γ- and δ-lactones from symmetrical hydroxy dicarboxylates and dihydroxy monocarboxylates. On a more general note, this study shows that prochiral selectivity can be achieved by enzymes in organic solvents and used for reactions that are not feasible in aqueous solutions where hydrolysis is the dominant reaction.

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Reaction of Diphenylmethylene with Carbon Dioxide: Matrix Isolation of Diphenyloxiranone

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Summary: The thermal or photochemical reaction of matrix-isolated diphenylmethylene and CO_2 produces the highly unstable diphenyloxiranone, which was characterized by IR spectroscopy, isotopic labeling, and subsequent photochemistry.

Sir: The reactivity of carbenes depends largely on the spin state from which the carbene reacts. At room temperature carbenes with triplet ground state frequently yield products from both the excited singlet state and the triplet ground state. If the singlet–triplet gap (ΔG_{ST}) is small, as in diphenylmethylene (1) (ΔG_{ST} = 4.2 kcal/mol),[1] singlet reactivity can arise from thermally equilibrated spin states. Griller explains the singlet reactivity of triplet carbenes by assuming an intersystem crossing (ISC) step during the course of the reaction.[1b] Singlet products are thus formed directly in a "spin forbidden" reaction from triplet carbenes and singlet substrates. To elucidate the role of spin states on chemical reactivity we have studied both "spin allowed" and "spin forbidden" carbene reactions at low temperature (20–50 K), where electronically excited states are not thermally populated if ΔG_{ST} is larger than a few kilocalories/mole.[2]

Here we report on the direct observation of the "spin forbidden" reaction of carbene 1 and CO_2 to give diphenyloxiranone (2). Indirect evidence for the formation of the highly unstable α-lactone 2[3] in the photoreaction of diphenyldiazomethane (3) and dry ice (fluorotrichloromethane as solvent) was given by Wheland and Bartlett.[7] Alternative routes to 2 are the ozonolysis[7] or autoxidation[8,9] of diphenylketene.

Table I. IR Data of Diphenylmethylene (1), Matrix Isolated in Ar Matrices with Various Amounts of CO_2 at 10 K (Wavenumbers in cm^{-1})

Ar	5% CO_2	Δ^a	10% CO_2	Δ^a	assignment[b]
3074.5 m	3074.9 m	+0.4	3074.5 m	+0.0	ν C–H
1479.6 m	1476.2 m	−3.4	1475.8 m	−3.8	
1466.6 w	1465.6 m	−1.0	1465.2 m	−1.4	ν C–C (19a)
1432.4 w	1433.3 w	−0.9	1433.3 w	−0.9	ν C–C (19b)
1066.9 w	1065.5 w	−1.4	1066.0 w	−0.9	β C–H (18a)
1020.2 m	1019.2 m	−1.0	1019.2 m	−1.0	ring (12)
891.4 w	892.4 w	+1.0	893.4 w	+2.0	τ C–H (17b)
743.4 s	747.8 s	+4.4	748.2 s	+4.8	τ C–H (11)
673.5 s	676.4 s	+2.9	–[c]		ϕ C–C (4)
565.0 w	565.0 m	+0.0	565.0 m	+0.0	

[a] Frequency shift in CO_2-doped matrices. [b] Approximate description, Wilson notation for vibrations of phenyl rings in brackets (see ref 2d). The band at 1282 cm^{-1} (ν_{as} C(Ph)–C(1)–C(Ph), ref 2d) is too weak to be observed in CO_2 doped matrices. [c] Not to be observed due to the very strong and broad CO_2 absorption at 652 cm^{-1}.

Photolysis of diazomethane 3 (λ = 543 nm, 10 K) in 0–10% CO_2-doped Ar or Xe matrices produces carbene 1 in high yields.[2d] As long as the matrix is kept at 10 K, no thermal reaction of 1 and CO_2 is observed even at high O_2 concentrations (10%). At these high CO_2 concentrations carbene 1 and CO_2 molecules are in direct contact, which causes a perturbation of the IR spectrum of 1 (Table I). Compared to the spectrum of 1 in pure Ar bands are shifted up to 4.8 cm^{-1} to higher or 3.8 cm^{-1} to lower frequencies. Perturbation of the relative band intensities is also observed for several bands.

When a CO_2-doped matrix is warmed to 35 K, IR bands[10] assigned to 1 slowly decrease and new bands appear. After 5-h annealing (10% CO_2 in Ar) at 35 K about 40% of 1 is converted to a new species, which is characterized by strong absorptions at 1890.4, 1877.8, and 699.1 cm^{-1} (Table II). In Xe matrices (5% O_2) 90% conversion is observed after 12-h annealing at 70 K. UV irradiation (λ > 220 nm) of the new species slowly produces carbon monoxide (2140.6 cm^{-1}) and benzophenone (4) (1663.8 cm^{-1}).

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(3) α-Lactones in general polymerize at low temperatures, and only two species with electron-attracting (ref 4) and additionally bulky (ref 5) substituents have some stability at room temperature. Several alkyl-substituted α-lactones, generated at 77 K by irradiation of peroxy malonates, have been shown to give polyesters at temperatures as low as −100 °C (ref 6).

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(10) IR spectra were recorded by using a Bruker IFS66 FT-IR spectrometer. The resolution was generally 1 cm^{-1}, spectra were recorded in the range 4000–500 cm^{-1}. The set up for matrix isolation is described in ref 2a and b.

0022-3263/89/1954-4265$01.50/0